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Variation in the diet of killer whales (*Orcinus orca*) at Marion Island, Southern Ocean

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Running head: Killer whale diet variation

ABSTRACT

Diet seems to be a key factor driving diversity and isolation among killer whale populations. Killer whales at Marion Island, Southern Ocean, have been observed preying on seals and penguins but are also know to depredate Patagonian toothfish from longline fishing vessels. However, their diet is poorly known especially when they occur offshore. We analysed carbon and nitrogen stable isotope ratios in 32 skin samples collected from 24 killer whales. Adult males showed higher $\delta^{15}N$ values than adult females or subadults indicating that they occupy a higher relative trophic level. There were not significant differences in $\delta^{13}C$ among eight social units, but $\delta^{15}N$ differed significantly and two social units which have been observed depredating Patagonian toothfish had higher $\delta^{15}N$ values. The inshore presence of killer whales at Marion Island was a significant predictor of their $\delta^{13}C$ values, but not of $\delta^{15}N$ values. This suggests some foraging north of Marion Island, potentially on lower trophic level prey. We also analysed tissue samples from seal, penguin and Patagonian toothfish prey and used available values for Antarctic fur seals. Results show that killer whales around Marion Island are apex predators, but that they do not feed exclusively on other high trophic level predators such as elephant seals, fur seals, and Patagonian toothfish. Killer whales had $\delta^{15}N$ values similar to those of Patagonian toothfish and adult male elephant seals, implying that the diet of killer whales at Marion Island includes some lower trophic level prey such as cephalopods or fishes.

Key words: predator; stable isotopes; carbon; $\delta^{13}C$; nitrogen; $\delta^{15}N$; foraging; trophic level

INTRODUCTION

Large, mobile apex predators have the potential to affect the structure and function of ecosystems through multiple pathways (Heithaus et al. 2008). However, a better understanding of the ecological roles and importance of predators requires detailed knowledge of their trophic interactions, which might be challenging in marine and wide-ranging predators such as cetaceans (Kiszka et al. 2015).

Killer whales (*Orcinus orca*) are apex predators with a broad diet including over 140 prey species, ranging in size from herring (*Clupea harengus*) to baleen whales (Ford 2009). Killer whale populations tend to specialise on taxonomic prey resources and the most well-known example of this is in the coastal northeast Pacific where three sympatric populations (or ecotypes) of killer whales – termed *transients*, *residents* and *offshores* – specialize on marine mammals or fish. In the Antarctic, it has been suggested that *type A* killer whales feed mainly on Antarctic minke whales (*Balaenoptera bonaerensis*), *large type B* killer whales eat mainly seals, *small type Bs* feed on penguins, and *type C* killer whales specialize on fishes (Pitman & Ensor 2003, Pitman & Durban 2010, 2012). These dietary differences are accompanied by differences in the ecotypes' social structure, morphology, acoustic behaviour, and movement patterns, with many ecotypes being genetically distinct (reviewed by de Bruyn et al. 2013). Various authors have proposed evolutionary mechanisms by which dietary specialization may promote genetic differentiation in killer whales (e.g., Moura et al. 2015 and references therein; although see Foote et al. 2013) and there seem to be some parallels in top predators such as wolves (*Canis lupus*) (Pilot et al. 2012), arctic foxes (*Alopex lagopus*) (Dalén et al. 2005) and bottlenose dolphins (*Tursiops* spp.) (Louis et al. 2014). In killer whales, these resource use patterns are culturally transmitted within matrilineal social units (see Riesch et al. 2012 for an overview).

However, there are suggestions of killer whale populations pursuing multiple categories of prey (reviewed by de Bruyn et al. 2013) and Baird (2002) suggested that dietary specialization in killer whales should also be related to environmental productivity, in keeping with optimal foraging theory. That is to say, in low productivity areas killer whales should be less likely to specialize, and diet breadth should decrease as the availability of the most profitable prey increases. However, other mechanisms may be responsible for specialization (Araújo et al. 2011) and there is a paucity of data from low latitudes and oceanic areas. It has been speculated that killer whales have played a role in population declines of some marine mammals and penguins (e.g., Springer et al. 2003, Ainley

et al. 2010), and their roles in ecosystems (e.g., Williams et al. 2004) has added impetus to dietary studies in this species.

In species where direct observation is often impractical, such as marine mammals, stable isotope analysis has become widely used to study diet (Newsome et al. 2010). Stable isotopes can be used as tracers in food webs because the biomolecular composition of the predator reflects the isotopic composition of the prey consumed in a predictable manner (DeNiro & Epstein 1976). The most commonly measured stable isotopes in foraging ecology are those of carbon (^{13}C:^{12}C) and nitrogen (^{15}N:^{14}N), reflecting foraging habitat and relative trophic position, respectively (Layman et al. 2012). An advantage of stable isotope analysis over other methods such as stomach content and faecal analysis is that it provides dietary information integrated over time (Dalerum & Angerbjörn 2005). However, stable isotope analysis typically cannot resolve prey identity as definitively as other methods and the actual diet may be difficult to ascertain (Tollit et al. 2010). Nevertheless, killer whale foraging ecology has been investigated using stable isotope analysis in the North Atlantic (McHugh et al. 2007, Foote et al. 2012, 2013), Arctic (Matthews & Ferguson 2013), North Pacific (Herman et al. 2005, Krahn et al. 2007a, 2007b, Newsome et al. 2009, Endo et al. 2014) and Antarctic (Krahn et al. 2008). No subantarctic killer whale stable isotope studies have been published.

The Prince Edward Islands – comprising Marion Island and the smaller Prince Edward Island – are an isolated pair of subantarctic islands in the Southern Ocean which provide a breeding and moulting site for millions of penguins and hundreds of thousands of seals (Ryan & Bester 2008). The abundance of penguins and seals attracts ~45 killer whales to the inshore waters of the islands annually (Reisinger et al. 2011a, Reisinger & de Bruyn 2014). These individuals have been classified into 9 social units, consisting of kin as well as non-kin (Reisinger 2015), although all individuals sampled share a single mitochondrial haplotype (Janse van Rensburg 2015). Social units regularly interact and dispersal of individuals between them has been recorded (Reisinger 2015). The Marion Island haplotype has been recorded among 13 haplotypes from 37 South African killer whales but no photographic identification matches have been made and the populations are significantly genetically different (Janse van Rensburg 2015). Marion Island killer whales have been directly observed preying on southern elephant seals, Subantarctic fur seals (*Arctocephalus tropicalis*), king penguins (*Aptenodytes patagonicus*), macaroni penguins (*Eudyptes chrysolophus*) and rockhopper

penguins (*E. chrysocome filholi*) (Reisinger et al. 2011c). Predation on Antarctic fur seals (*Arctocephalus gazella*) has not been documented, but is likely to occur.

Killer whales also depredate Patagonian toothfish (*Dissostichus eleginoides*) from two licenced longline fishing vessels which operate in the vicinity of the islands (Williams et al. 2009). It is unknown what proportion of Marion Island killer whales depredate toothfish, but nine individuals which depredate toothfish from fishing vessels around the Crozet Islands (~1,000 km due east of the Prince Edward Islands) are also sighted inshore at Marion Island (Reisinger & de Bruyn 2014, Tixier et al. 2014).

Killer whales are most abundant at Marion Island from September to December, with a secondary peak in April and May, which coincides with the breeding and moulting period of seals and penguins (Reisinger et al. 2011c). The abundance of seals and penguins inshore at Marion Island may change 10-fold or more within a year (e.g., Oosthuizen et al. 2012) and this is expected to cause a seasonal distribution and/or diet shift in killer whales in response.

All killer whale studies at Marion Island to date have relied on direct, shore-based observations of predation, which have allowed limited inferences about the diet of killer whales in this population, and about any intra-population or temporal dietary variations. We therefore used stable isotope analysis of carbon and nitrogen to provide information about the unseen foraging ecology of killer whales at Marion Island. More specifically, we investigated 1) whether there is any evidence for diet variation among individuals, social units and age- or sex-classes; and 2) if there is any variation in killer whale stable isotope values linked to their inshore presence, which would indicate diet and/or foraging distribution changes related to changing resource availability. In addition, we provide some inference regarding the diet of Marion Island killer whales and the first concurrent isotopic description of seals, penguins, and Patagonian toothfish at Marion Island. Given the common ancestry of Marion Island killer whales and the relatively dynamic nature of their social organization (Reisinger 2015), we hypothesized that there should not be significant dietary differentiation among social units in this population. Secondly, given the seasonal fluctuations in prey distribution and abundance at Marion Island (Reisinger et al. 2011c) we hypothesized that killer whales should change their diet seasonally and/or forage in a different environment.

METHODS

Sample collection and preparation

Skin biopsy samples were remotely collected from live killer whales at Rockhopper Bay and Transvaal Cove, Marion Island (46.873° S 37.859° E) from August 2011 to May 2013 (Table 1). Tissue samples were obtained using sterilized stainless steel biopsy tips (25 mm long, 7 mm inside diameter) attached to bolts which were fired from a 68 kg draw weight recurve crossbow (Barnett Panzer V, Barnett Outdoors, LLC, Tarpon Springs, Florida, USA). The region on the body directly below the dorsal fin was targeted. Individuals were identified from photographs, video or by eye (later confirmed from photographs) following Reisinger et al. (2011a) and Reisinger and de Bruyn (2014). The social unit designation follows Reisinger (2015). Sampling procedures, as well as the effects of sampling on killer whales, are discussed in detail in Reisinger et al. (2014). Samples were stored at -80°C (without preservatives) within 30 minutes of collection. We lyophilized the samples for 24 hours before they were transported. Before homogenising samples, we separated the skin from the blubber portion.

The presence of lipids in tissues introduces variation in $\delta^{13}C$ values, because the lipid content of organisms is heterogeneous and lipids have lower $\delta^{13}C$ values (DeNiro & Epstein 1977, Post et al. 2007). Accounting for lipids is important in lipid-rich tissues such as cetacean skin and blubber (Ryan et al. 2012). We took two aliquots from each skin sample: one aliquot was analysed without extracting lipids and the second aliquot was lipid extracted. To extract lipids we added 8 ml dicloromethane:methanol (3:1, volume:volume) to each sample and sonicated them for 15 mins. We then centrifuged samples at 3,000 rpm for 10 mins and discarded the supernatant. We repeated these steps twice more (i.e., three times in total). Next, we added distilled water, agitated the samples and sonicated them for 15 mins. We centrifuged the sample at 3,000 rpm for 10 mins and discarded the supernatant. After repeating these latter steps (i.e., twice in total) and then oven-drying the samples at 50°C for 48 hours, we weighed 0.4–0.5 mg sample aliquots into tin capsules for carbon and nitrogen stable isotope ratio analysis. Further correction was unnecessary as almost all atomic carbon:nitrogen (C:N) ratios met the ~4.1 threshold (C:N mass ratio of 3.5) recommended by Post et al. (2007). Because lipid extraction altered $\delta^{15}N$ values as well as correcting $\delta^{13}C$ values (see Supplementary Materials), we used lipid extracted $\delta^{13}C$ values with non lipid extracted $\delta^{15}N$ values

for further analysis, as recommended by Ryan et al. (2012). Hereafter, unless stated otherwise, killer $\delta^{13}C$ values are from lipid extracted skin, and $\delta^{15}N$ values are from non lipid extracted skin.

We used $\delta^{13}C$ and $\delta^{15}N$ values for all prey species recorded in the diet of Marion Island killer whales and all species were sampled at or near (in the case of Patagonian toothfish) Marion Island (Table 2 and Supplementary Tables S1–S3). While we attempted to match killer whale and prey samples closely in time, this was not always possible. Prey samples were collected in four years, and in seven calendar months (November-May) (Supplementary Tables S1-S3). Marked inter- or intra-annual variation in prey foraging behaviour or oceanographic conditions could thus influence our representation of the killer whale prey field. Sample collection and preparation is detailed in the Supplementary Materials. Additionally, we used $\delta^{13}C$ and $\delta^{15}N$ values for whole blood samples collected from 35 adult female Antarctic fur seals at Marion Island, reported in Walters (2014). The mean $\delta^{13}C$ value of these was -21.7 ± 0.5‰ and the mean $\delta^{15}N$ value was 11.2 ± 0.3‰.

Stable isotope analysis

We analysed killer whale, penguin and seal tissue at the Stable Isotope Biogeochemistry Laboratory, Durham University, UK. We measured $\delta^{13}C$ and $\delta^{15}N$ using a Thermo-Finnigan Delta V Advantage isotope ratio mass spectrometer (Thermo Fisher Scientific, Bremen, Germany), coupled with a Costech ECS 4010 Elemental Analyser. Tin capsules containing samples were sequentially combusted in the elemental analyser; the resulting gases were passed through a gas chromatography column for separation and then measured in succession by the isotope ratio mass spectrometer. We monitored data accuracy by analysing in-house standards which are stringently calibrated against international standards (e.g., USGS 24 and 40, IAEA N1, N2 and 600 for $\delta^{15}N$; USGS 40, IAEA 600, SPAR for $\delta^{13}C$). Analytical uncertainty for δ values was typically ± 0.1‰ for replicate analyses of the international standards and typically < 0.2‰ on replicate sample analysis for both $\delta^{13}C$ and $\delta^{15}N$.

We analysed Patagonian toothfish samples at the Mammal Research Institute, University of Pretoria, South Africa. Tin cups containing the samples were combusted at 1020°C in an elemental analyser (Flash 1112 Series, Thermo Fisher Scientific). We determined $\delta^{13}C$ and $\delta^{15}N$ values using a Delta V Plus isotope ratio mass spectrometer (Thermo Fisher Scientific). We placed an in-house standard

(Merck gel) and blank after every 10 samples to ensure reproducibility. Reproducibility in $\delta^{13}C$ and $\delta^{15}N$ was ± 0.2‰.

We report stable isotope ratio values (^{13}C:^{12}C and ^{15}N:^{14}N) relative to the standards Vienna Peedee Belemnite (for carbon) and air (for nitrogen), expressed in parts per thousand (‰).

Statistical analysis

We performed statistical tests in the R environment (R Core Team 2013). We tested normality using a Shapiro-Wilk test, before applying an appropriate parametric (paired *t*-test, ANOVA) or non-parametric test (Welch's *t*-test, Mann-Whitney *U* test, Kruskal-Wallis test). To investigate the effect of class and social unit on killer whale δ values, while taking into account multiple sampling of individuals, we fitted linear mixed effects models using the *lme4* (Bates et al. 2014) and *MuMIn* (Barton 2013) packages in R. We included class and social unit as fixed terms, and individual as a random term. Fixed effects were assessed by comparing the null models to those with the fixed effect in question, using likelihood ratio tests. Where necessary, these were followed by a post-hoc Tukey's Honest Significant Difference (HSD) test, using the *multcomp* package in R (Hothorn et al. 2008).

To assess the influence which the inshore presence of killer whales had on δ values, we calculated a population level sighting rate in the 75 days prior to each biopsy sampling event. We assumed that the full profile skin samples we collected would represent diet integrated over the preceding 70-75 days (Hicks et al. 1985, St. Aubin et al. 1990). The sighting rate was calculated as the number of sightings of killer whales during shore based dedicated killer whale search sessions at Marion Island (2008–2013; 1,285 sightings in 5,498 hours) (see Reisinger et al. 2011a, 2011c and 2014 for further details). The sighting rate was included as a fixed effect in linear mixed effects models, as for class and social unit (above).

Further, all models were compared using small sample size Akaike Information Criterion (AIC_c) and goodness of fit was assessed using the conditional coefficient of determination ($R^2_{GLMM(c)}$) (Nakagawa & Schielzeth 2013).

To visualize comparisons among classes and social units, for groups with more than two samples we calculated the small sample size corrected Standard Ellipse Area (SEA_c) (Jackson et al. 2011) in the *SIAR* package (Parnell et al. 2010, Parnell & Jackson 2013) in R. The SEA_c comprises approximately 40% of the data points, and can be interpreted as a representation of standard deviation for bivariate data (Jackson et al. 2011).

Inferring prey

As prey tissues are assimilated into a consumer's body following ingestion, δ values change due to isotopic fractionation. $\delta^{13}C$ values change little (~0.75 ‰), while $\delta^{15}N$ values show greater change (~2.75 ‰), but these values vary with factors including age, body size and diet quality (Caut et al. 2009). These trophic discrimination factors (Δ values) are tissue- and species-specific, and are typically measured in controlled feeding studies (e.g., Hobson et al. 1996, Browning et al. 2014). Three studies (Caut et al. 2011, Browning et al. 2014, Giménez et al. 2016) have estimated trophic discrimination factors in cetaceans experimentally. We used values reported in Browning et al. (2014) ($\Delta^{13}C_{diet-skin}$ = 1.1 ± 0.6% and $\Delta^{15}N_{diet-skin}$ = 2.1 ± 0.5%), estimated for bottlenose dolphins, to visualize the potential prey "mixing space" for killer whales in this study.

Different tissues have different diet-tissue discrimination factors and are not directly comparable without adjustment. To facilitate comparisons among prey and to visualize the putative prey of killer whales, we therefore adjusted $\delta^{13}C$ and $\delta^{15}N$ values of prey blood (fur seals and penguins) and hair (elephant seals) to represent muscle (Table 3), which we assumed would make up the largest proportion of protein assimilated by killer whales. We calculated adjustment values as the difference between the δ values for the two tissues concerned, measured in controlled feeding studies (Hobson et al. 1996, Evans Ogden et al. 2004) (Table 3). Unless otherwise specified, values for prey hereafter are adjusted values which represent muscle (Table 3). Our simplistic, but necessary, adjustment should be interpreted cautiously as our adjustment is approximate and tissues differ in a number of respects which we could not take into account, such as isotopic turnover rate.

We attempted to use Bayesian stable isotope mixing models (Parnell et al. 2013) to estimate the proportion of each prey type in the diet of Marion Island killer whales. However, the caveats and assumptions involved in their use — particularly regarding the accuracy of available diet-tissue discrimination factors and the assumption that all potential prey are sampled — (Bond & Diamond 2011, Parnell et al. 2013, Phillips et al 2014) made them an unsatisfactory tool for this study, thus precluding inference about proportional diet composition.

RESULTS

The mean $\delta^{13}C$ value in lipid extracted killer whale skin samples was -18.6 ± 0.4‰. Mean $\delta^{15}N$ value in non lipid extracted skin samples was 12.3 ± 0.6‰.

Skin $\delta^{13}C$ values were not significantly different among classes (χ^2 = 2.02, *df* = 2, *p* = 0.364) or among social units (χ^2 = 16.68, *df* = 9, *p* = 0.054). There was a significant among-class difference in $\delta^{15}N$ (χ^2 = 7.74, *df* = 2, *p* = 0.021) – adult males had significantly higher values than adult females (*p* = 0.006) (Figure 1). The difference among social units was also significant (χ^2 = 25.62, *df* = 9, *p* = 0.002); specifically, differences between several social units and Unit D showed the strongest differentiation (Supplementary Table S5).

We compared δ values for individuals which have been observed interacting with longline fishing vessels around the Crozet Islands (social units D and G; 5 samples from 4 individuals; Table 1) with those only observed at Marion Island. Skin $\delta^{13}C$ values did not differ significantly (Welch two sample t-test, *t* = -0.478, *df* = 5.23, *p* = 0.696) but $\delta^{15}N$ values did (*t* = -6.77, *df* = 9.92, *p* < 0.001). Killer whales sighted around Crozet had mean $\delta^{15}N$ values of 13.2 ± 0.3 ‰, while mean $\delta^{15}N$ for Marion individuals was 12.1 ± 0.5 ‰.

Sighting rate of killer whales in the 75 days prior to sampling was a significant predictor of $\delta^{13}C$ values (χ^2 = 22.71, *df* = 15, *p* = 0.030). Sighting rate was not a significant predictor of $\delta^{15}N$ values (χ^2 = 3.14, *df* = 4, *p* = 0.076), but models containing sighting rate were ranked above the null model according to AIC_c (Table 4). $\delta^{13}C$ values decreased slightly with increased sighting rates, and $\delta^{15}N$ values showed the opposite pattern (Figure 2). In both cases, however, the effect size was relatively

small: based on the equivalent linear models, predicted change in $\delta^{13}C$ over the observed sighting rates was -0.5‰, and that for $\delta^{15}N$ was +0.7‰.

Overall, the model containing only sighting rate was the optimal for $\delta^{13}C$, but the variance explained was low ($R^2_{GLMM(c)} = 0.189$) (Table 4). For $\delta^{15}N$, the optimal model contained class and sighting rate, with higher variance explained ($R^2_{GLMM(c)} = 0.467$) (Table 4).

Prey

There were significant differences among prey $\delta^{13}C$ values (ANOVA, $F = 23.2$, $df = 9$, $p < 0.001$) and among prey $\delta^{15}N$ values (ANOVA, $F = 67.0$, $df = 9$, $p < 0.001$), the latter showing a greater range of variation (Figure 3a). A post-hoc multiple comparisons using adjusted values (Tukey's HSD) is shown in Supplementary Table S4 and more detailed results are presented, and discussed, in the supplementary materials. Mean killer whale $\delta^{15}N$ (12.3 ± 0.6‰) was similar to, but slightly lower than, those of adult male southern elephant seals (12.7 ± 0.6‰) and Patagonian toothfish (13.3 ± 1.1‰) (Figure 3a). Mean killer whale $\delta^{13}C$ (-18.6 ± 0.4‰) was higher than that of all other prey, the nearest being southern elephant seal adult males (-18.3 ± 1.0‰) (Figure 3a). After subtracting discrimination factors from killer whale δ values, $\delta^{13}C$ values were similar to those of southern elephant seal adult males, and $\delta^{15}N$ values were slightly below those of king penguins (Figure 3b). However, no prey values were close to the adjusted killer whale values in bivariate space (Figure 3b).

DISCUSSION

Killer whale diet

We show that killer whales around Marion Island do not feed exclusively on other high trophic level predators such as elephant seals, fur seals, and Patagonian toothfish. Killer whales from Marion Island have $\delta^{15}N$ values similar to those of Patagonian toothfish and adult male elephant seals.

The diet of adult male elephant seals at Marion Island is unknown, but at other locations their diet consists largely of cephalopods and fishes (Supplementary Materials). The diet of toothfish sampled near Marion Island was dominated by cephalopods and Myctophids (Pakhomov et al. 2006).

Cephalopods are an important element in the Southern Ocean marine ecosystem and are major components of the diets of toothed whales, seals, penguins, and large fishes (Rodhouse 2013). Killer whales feed on cephalopods elsewhere (see references in Reisinger et al. 2015) and Hanson and Walker (2014) suggest that cephalopods represent a previously underestimated component of *transient* (mammal-hunting) killer whales' diets. Reisinger et al. (2015) postulated that the depths (up to 499 m and 767 m) and diel variation in the dive behaviour of two killer whales at Marion Island was related to predation on prey such as cephalopods. However, published values for the cephalopod community at the Crozet Islands (Guerreiro et al. 2015) did not lie near adjusted killer whale vales in bivariate space (cf. Figure 3b). Various fish species could also be considered prey based on their isotope ratio values (Pakhomov et al. 2006), but are too small to be energetically worthwhile prey. Potential prey species north of Marion Island were unavailable, and the lack of suitable prey values within the potential solution polygon of the adjusted killer whale values in bivariate space (Figure 3b), suggests that potential prey species were missing. Nevertheless, $\delta^{15}N$ values confirm the consumption of lower trophic level prey (such as cephalopods and fishes), by Marion Island killer whales.

Killer whale class, social unit and temporal variability in diet

The relationship between killer whale sighting rate and δ values indicates that there is some variation in the foraging area, and possibly diet, of killer whales through the annual cycle. This is likely driven by the seasonal changes in prey abundance at Marion Island. The decrease of $\delta^{13}C$ values with increased inshore sighting rate is consistent with a shift from a diet including some prey consumption north of Marion Island to prey consumed primarily around Marion Island. There is a latitudinal gradient in baseline $\delta^{13}C$ values in the Southern Ocean (Trull & Armand 2001) and consequently consumer $\delta^{13}C$ values will reflect the latitudinal provenance of their diet (Jaeger et al. 2010). Based on Jaeger et al.'s (2010) regression of wandering albatross plasma (*Diomedea exulans*) $\delta^{13}C$ values against foraging latitude, and ignoring any effect of changing trophic level, our 0.5‰ predicted change in $\delta^{13}C$ would correspond to a 1.6° latitudinal shift in carbon provenance, a result corroborated by satellite tracking data of killer whale from Marion Island, showing restricted movement over seamounts to the north of the islands by some individuals (Reisinger et al. 2015). However, because $\delta^{13}C$ values also increase with trophic level (~0.75‰), the size of this effect would be masked by the potential concomitant increase in prey trophic level (signalled by increasing $\delta^{15}N$ values – Figure 2 and below) and the inferred latitudinal shift could be larger. Two of nine killer whales satellite-tracked from Marion Island moved north of the subtropical front. While their

movements were rapid and directed, with no restricted movement patterns suggesting foraging behaviour (Reisinger et al. 2015), a different foraging mode is possible.

Although the increase in killer whale skin $\delta^{15}N$ with increased sighting rate was not significant, sighting rate was included in our best model predicting $\delta^{15}N$ values. We interpret the increase in $\delta^{15}N$ values as greater consumption of higher trophic level prey, notably southern elephant seals, when killer whales are present inshore. The decreased abundance of seals and penguins inshore primarily during the austral winter will result in increased search times for these prey, and should consequently lower their profitability for killer whales. Killer whales can respond by altering their diet (including more prey types, or switching to other prey types), by hunting in different habitats, or a combination of these. The relationship between δ values and sighting rate, in conjunction with movement and dive data (Reisinger et al. 2015), suggests a combination of these strategies. However, the effect size is small and we require more killer whale samples throughout the year to rigorously test to what degree foraging behaviour changes.

Although there were marginally significant differences in $\delta^{15}N$ values among social units, there was no obvious pattern of differentiation, evidence for broadly similar diets among social units at Marion Island. There was no evidence for divergent ecological specialization. The apparently similar resource-use strategies in this population may be related to the population's overall genetic relatedness and frequent social contact (Reisinger 2015).

Individuals which interacted with the Patagonian toothfish longline fisheries around the Crozet Islands showed $\delta^{15}N$ values about 1 ‰ higher than individuals which were only sighted at Marion Island, suggesting that this high trophic level prey may have had a detectable influence on killer whale $\delta^{15}N$ values and that Marion Island killer whales may depredate Patagonian toothfish to a lesser degree. This might be expected given that only two vessels operate legally in the vicinity of Marion Island (with zero estimated illegal, unregulated and unreported fishing), compared to seven around the Crozet Islands (Tixier et al. 2015).

However, three of the five samples from Units D and G were from adult males, and adult males had significantly higher $\delta^{15}N$ values than other classes. Thus the high sample proportion from adult males

in the comparison of social units D and G to other units is a confounding factor, and could provide an alternative explanation to Patagonian toothfish depredation. The three samples from these toothfish depredating males may also have caused the significantly higher $\delta^{15}N$ values for all males compared to adult females and subadults, mean $\delta^{15}N$ for the three samples from two depredating males was 13.3 ‰ and that for the three non-depredating males was 12.7 ‰. Disentangling the effect is difficult with such a small number of samples, but the ranking of linear models using AIC_c (Table 4) suggests that class is a more parsimonious explanatory variable for $\delta^{15}N$ than social unit.

Since killer whales hunt cooperatively and often share prey with members of their social unit (e.g., Hoelzel 1991, Ford & Ellis 2006), the higher $\delta^{15}N$ values for adult males are unexpected. Newsome et al. (2009) found the same among North Pacific *transient* killer whales and suggested that adult male killer whales may sometimes forage on their own at a higher trophic level, or that trophic discrimination factors become larger when growth rate slows (as less dietary protein is used directly for tissue synthesis to sustain growth). Endo et al. (2014) report a positive correlation between $\delta^{15}N$ values and body length. Given the dynamic social organization of Marion Island killer whales (Reisinger 2015; discussed above), it is possible that males do forage on their own, but we consider it unlikely that lone individuals successfully hunt large prey such as elephant seals. It may instead be that adult males take less lower trophic level prey if they forage on their own, or take more high trophic level prey (apart from adult male elephant seals), such as Subantarctic fur seals or Patagonian toothfish.

Conclusions and further research

We show that killer whales are indeed apex predators in the Marion Island marine ecosystem, with mean $\delta^{15}N$ values similar to adult male southern elephant seals and Patagonian toothfish. However $\delta^{15}N$ values in killer whales were not high enough to suggest that they prey exclusively on high trophic level prey (seals and toothfish). Cephalopods, and perhaps some fishes, represent possible prey in addition to seals, penguins and toothfish. Killer whales in the Southern Ocean may thus have broader ecosystem impacts than previously suggested. The inclusion of lower trophic level prey is likely driven by seasonal changes in the abundance of seals and penguins. There was no strong differentiation in trophic ecology among social units, but individuals from two social units, known to depredate Patagonian toothfish around the Crozet Islands, had slightly higher $\delta^{15}N$ values. Adult

male killer whales appeared to occupy a slightly higher relative trophic level than females and subadults.

The lack of accurate trophic discrimination factors, and possibly missing δ values for unknown prey of killer whales precluded diet reconstructions using mixing models. Sampling all possible prey is rarely possible, especially in a versatile predator such as the killer whale, but we suggest further work to characterize potential cephalopod and fish prey around Marion Island. Accurate, species-specific trophic discrimination factors will continue to be rare for cetaceans. Compound specific stable isotope analysis or fatty acid analysis can be used as additional, and perhaps more informative, dietary tracers. We recommend further biopsy sampling so that more robust inferences can be made, but the 24 individuals we sampled already represent approximately half of the Marion Island population of killer whales.

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REFERENCES

Ainley D, Ballard G, Blight LK, Ackley S, Emslie ST, Lescroel A, Olmastroni S, Townsend SE, Tynan CT, Wilson P, Woehler E (2010) Impacts of cetaceans on the structure of Southern Ocean food webs. Mar Mammal Sci 26:482–498

Araújo MS, Bolnick DI, Layman CA (2011) The ecological causes of individual specialisation. Ecol Lett 14:948–958

Baird RW (2002) Killer whales of the world— natural history and conservation. Voyageur Press, Stillwater, Minnesota

Barton K (2013) MuMIn: Multi-model Inference. R Package Version 1.9.13. http://CRAN.R-project.org/package=MuMIn.

Bates D, Maechler M, Bolker B, Walker S (2014) lme4: Linear mixed-effects models using Eigen and S4. R package version 1.1-6. http://CRAN.R-project.org/package=lme4

Bond A, Diamond A (2011) Recent Bayesian stable-isotope mixing models are highly sensitive to variation in discrimination factors. Ecol Appl 21:1017–1023

Browning NE, Dold C, I-Fan J, Worthy GAJ (2014) Isotope turnover rates and diet-tissue discrimination in skin of ex situ bottlenose dolphins (*Tursiops truncatus*). J Exp Biol 217:214–21

Caut S, Angulo E, Courchamp F (2009) Variation in discrimination factors (Δ 15 N and Δ 13 C): the effect of diet isotopic values and applications for diet reconstruction. J Appl Ecol 46:443–453

Cherel Y, Ducatez S, Fontaine C, Richard P, Guinet C (2008) Stable isotopes reveal the trophic position and mesopelagic fish diet of female southern elephant seals breeding on the Kerguelen Islands. Mar Ecol Prog Ser 370:239–247

Dalén L, Fuglei E, Hersteinsson P, Kapel CMO, Roth JD, Samelius G, Tannerfeldt M, Angerbjörn A (2005) Population history and genetic structure of a circumpolar species: The arctic fox. Biol J Linn Soc 84:79–89

Dalerum F, Angerbjörn A (2005) Resolving temporal variation in vertebrate diets using naturally occurring stable isotopes. Oecologia 144:647–58

De Bruyn PJN, Tosh CA, Terauds A (2013) Killer whale ecotypes: is there a global model? Biol Rev Camb Philos Soc 88:62–80

DeNiro MJ, Epstein S (1976) You are what you eat (plus a few per mil): the carbon isotope cycle in food chains. Geological Society of America Abstracts with Programs 8:834–835.

DeNiro MJ, Epstein S (1977) Mechanism of carbon isotope fractionation associated with lipid synthesis. Science 197:261-26

Endo T, Kimura O, Sato R, Kobayashi M, Matsuda A, Matsuishi T, Haraguchi K (2014) Stable isotope ratios of carbon, nitrogen and oxygen in killer whales (*Orcinus orca*) stranded on the coast of Hokkaido, Japan. Mar Pollut Bull 86:238–243

Evans Ogden LJ, Hobson KA, Lank DB (2004) Blood isotopic ($\delta^{13}C$ and $\delta^{15}N$) turnover and diet-tissue fractionation factors in captive Dunlin (*Calidris alpina pacifica*). Auk 121:170–177

Foote AD, Newton J, Avila-Arcos MC, Kampmann M-L, Samaniego JA, Post K, Rosing-Asvid A, Sinding M-HS, Gilbert MTP (2013) Tracking niche variation over millennial timescales in sympatric killer whale lineages. Proc R Soc B Biol Sci 280:20131481–20131481

Foote AD, Vester H, Víkingsson GA, Newton J (2012) Dietary variation within and between populations of northeast Atlantic killer whales, *Orcinus orca* , inferred from $\delta^{13}C$ and $\delta^{15}N$ analyses. Mar Mammal Sci 28:E472–E485

Ford JKB (2009) Killer Whale: *Orcinus orca*. In: William FP, Bernd W, Thewissen JGM (eds) Encyclopedia of Marine Mammals (Second Edition). Academic Press, London, p 650–657

Ford JKB, Ellis GM (2006) Selective foraging by fish-eating killer whales *Orcinus orca* in British Columbia. Mar Ecol Prog Ser 316:185–199

Giménez J, Ramírez F, Almunia J, Forero MG, de Stephanis R (2016) From the pool to the sea: Applicable isotope turnover rates and diet to skin discrimination factors for bottlenose dolphins (*Tursiops truncatus*). J Exp Mar Biol Ecol 475:54-61

Hanson B, Walker WA (2014) Trans-Pacific consumption of cephalopods by North Pacific Killer Whales (*Orcinus orca*). Aquat Mamm 40:274–284

Heithaus MR, Frid A, Wirsing AJ, Worm B (2008) Predicting ecological consequences of marine top predator declines. Trends Ecol Evol 23:202-210

Herman DP, Burrows DG, Wade PR, Durban JW, Matkin CO, Leduc RG, Barrett-Lennard LG, Krahn MM (2005) Feeding ecology of eastern North Pacific killer whales *Orcinus orca* from fatty acid, stable isotope, and organochlorine analyses of blubber biopsies. Mar Ecol Prog Ser 302:275–291

Hicks BD, St. Aubin DJ, Geraci JR, Brown WR (1985) Epidermal growth in the bottlenose dolphin, *Tursiops truncatus.* J Invest Dermatol 85:60–63

Hobson KA, Schell DM, Renouf D, Noseworthy E (1996) Stable carbon and nitrogen isotopic fractionation between diet and tissues of captive seals: implications for dietary reconstructions involving marine mammals. Can J Fish Aquat Sci 53:528–533

Hoelzel AR (1991) Killer whale predation on marine mammals at Punta Norte, Argentina; food sharing, provisioning and foraging strategy. Behav Ecol Sociobiol 29:197–204

Hothorn T, Bretz F, Westfall P (2008) Simultaneous inference in general parametric models. Biometrical J 50:346–363

Jackson AL, Inger R, Parnell AC, Bearhop S (2011) Comparing isotopic niche widths among and within communities: SIBER – Stable Isotope Bayesian Ellipses in R. J Anim Ecol 80:595–602

Jaeger A, Lecomte VJ, Weimerskirch H, Richard P, Cherel Y (2010) Seabird satellite tracking validates the use of latitudinal isoscapes to depict predators' foraging areas in the Southern Ocean. Rapid Commun Mass Spectrom 24:3456–3460

Janse van Rensburg C (2015) Molecular ecology of killer whales (*Orcinus orca*) from Marion Island and South Africa. MSc dissertation. University of Pretoria, Pretoria

Kiszka JJ, Heithaus MR, Wirsing AJ (2015) Behavioural drivers of the ecological roles and importance of marine mammals. Mar Ecol Prog Ser 523:267-281

Krahn MM, Hanson MB, Baird RW, Boyer RH, Burrows DG, Emmons CK, Ford JKB, Jones LL, Noren DP, Ross PS, Schorr GS, Collier TK (2007a) Persistent organic pollutants and stable isotopes in biopsy samples (2004/2006) from Southern Resident killer whales. Mar Pollut Bull 54:1903–11

Krahn MM, Herman DP, Matkin CO, Durban JW, Barrett-Lennard LG, Burrows DG, Dahlheim ME, Black N, LeDuc RG, Wade PR (2007b) Use of chemical tracers in assessing the diet and foraging regions of eastern North Pacific killer whales. Mar Environ Res 63:91–114

Krahn MM, Pitman RL, Burrows DG, Herman DP, Pearce RW (2008) Use of chemical tracers to assess diet and persistent organic pollutants in Antarctic Type C killer whales. Mar Mammal Sci 24:643–663

Layman CA, Araujo MS, Boucek R, Hammerschlag-Peyer CM, Harrison E, Jud ZR, Matich P, Rosenblatt AE, Vaudo JJ, Yeager LA, Post DM, Bearhop S (2012) Applying stable isotopes to examine food-web structure: an overview of analytical tools. Biol Rev Camb Philos Soc 87:545–62

Louis M, Fontaine MC, Spitz J, Schlund E, Dabin W, Deaville R, Caurant F, Cherel Y, Guinet C, Simon-Bouhet B (2014) Ecological opportunities and specializations shaped genetic divergence in a highly mobile marine top predator. Proc R Soc B Biol Sci 281:20141558

Matthews CJD, Ferguson SH (2013) Spatial segregation and similar trophic-level diet among eastern Canadian Arctic/north-west Atlantic killer whales inferred from bulk and compound specific isotopic analysis. J Mar Biol Assoc United Kingdom 94:1343–1355

McHugh B, Law RJ, Allchin CR, Rogan E, Murphy S, Foley MB, Glynn D, McGovern E (2007) Bioaccumulation and enantiomeric profiling of organochlorine pesticides and persistent organic pollutants in the killer whale (*Orcinus orca*) from British and Irish waters. Mar Pollut Bull 54:1724–31

Moura AE, Kenny JG, Chaudhuri RR, Hughes MA, Reisinger RR, de Bruyn PJN, Dahlheim ME, Hall N, Hoelzel AR (2015) Phylogenomics of the killer whale indicates ecotype divergence in sympatry. Heredity 114:48–55

Nakagawa S, Schielzeth H (2013) A general and simple method for obtaining R^2 from generalized linear mixed-effects models. Methods Ecol Evol 4:133–142

Newsome SD, Clementz MT, Koch PL (2010) Using stable isotope biogeochemistry to study marine mammal ecology. Mar Mammal Sci 26:509–572

Newsome SD, Etnier M, Monson D, Fogel M (2009) Retrospective characterization of ontogenetic shifts in killer whale diets via $\delta^{13}C$ and $\delta^{15}N$ analysis of teeth. Mar Ecol Prog Ser 374:229–242

Oosthuizen WC, de Bruyn PJN, Bester MN (2012) Unmarked individuals in mark–recapture studies: Comparisons of marked and unmarked southern elephant seals at Marion Island. Austral Ecol 35:556–568

Pakhomov EA, Bushula T, Kaehler S, Watkins BP, Leslie RW (2006) Structure and distribution of the slope fish community in the vicinity of the sub-Antarctic Prince Edward Archipelago. J Fish Biol 68:1834–1866

Parnell AC, Inger R, Bearhop S, Jackson AL (2010) Source partitioning using stable isotopes: coping with too much variation. PLoS One 5:e9672

Parnell AC, Jackson AL (2013) siar: Stable Isotope Analysis in R. R package version 4.2. http://CRAN.R-project.org/package=siar

Parnell AC, Phillips DL, Bearhop S, Semmens BX, Ward EJ, Moore JW, Jackson AL, Grey J, Kelly DJ, Inger R (2013) Bayesian stable isotope mixing models. Environmetrics 24: 387–399

Phillips DL, Inger R, Bearhop S, Jackson AL, Moore JW, Parnell AC, Semmens BX, Ward EJ (2014) Best practices for use of stable isotope mixing models in food-web studies. Can J Zool 92:823–835

Pilot M, Jedrzejewski W, Sidorovich VE, Meier-Augenstein W, Hoelzel AR (2012) Dietary differentiation and the evolution of population genetic structure in a highly mobile carnivore. PLoS One 7:1–11

Pitman RL, Durban JW (2010) Killer whale predation on penguins in Antarctica. Polar Biol 33:1589–1594

Pitman RL, Durban JW (2012) Cooperative hunting behavior, prey selectivity and prey handling by pack ice killer whales (*Orcinus orca*), type B, in Antarctic Peninsula waters. Mar Mammal Sci 28:16–36

Pitman RL, Ensor P (2003) Three forms of killer whales (*Orcinus orca*) in Antarctic waters. J Cetacean Res Manag 5:131–139

Post DM, Layman CA, Arrington DA, Takimoto G, Quattrochi J, Montaña CG (2007) Getting to the fat of the matter: models, methods and assumptions for dealing with lipids in stable isotope analyses. Oecologia 152:179–89

R Core Team (2013) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/

Reisinger RR (2015) Social organisation and foraging behaviour of killer whales (*Orcinus orca*) at sub-Antarctic Marion Island. PhD thesis, University of Pretoria, Pretoria

Reisinger RR, de Bruyn PJN (2014) Marion Island killer whales: 2006-2013. Mammal Research Institute, University of Pretoria, doi:10.6084/m9.figshare.971317

Reisinger RR, de Bruyn PJN, Bester MN (2011a) Abundance estimates of killer whales at subantarctic Marion Island. Aquat Biol 12:177–185

Reisinger RR, de Bruyn PNJ, Tosh CA, Oosthuizen WC, Mufanadzo NT, Bester MN (2011c) Prey and seasonal abundance of killer whales at sub-Antarctic Marion Island. African J Mar Sci 33:99–105

Reisinger RR, Keith M, Andrews RD, de Bruyn PJN (2015) Movement and diving of killer whales (*Orcinus orca*) at a Southern Ocean archipelago. J Exp Mar Biol Ecol 473:90–102

Reisinger RR, Oosthuizen WC, Péron G, Cory Toussaint D, Andrews RD, de Bruyn PJN (2014) Satellite tagging and biopsy sampling of killer whales at Subantarctic Marion Island: effectiveness, immediate reactions and long-term Responses. PLoS One 9:e111835

Riesch R, Barrett-Lennard LG, Ellis GM, Ford JKB, Deecke VB (2012) Cultural traditions and the evolution of reproductive isolation: ecological speciation in killer whales? Biol J Linn Soc 106:1–17

Rodhouse PGK (2013) Role of squid in the Southern Ocean pelagic ecosystem and the possible consequences of climate change. Deep Sea Res Part II Top Stud Oceanogr 95:129–138

Ryan C, McHugh B, Trueman CN, Harrod C, Berrow SD, O'Connor I (2012) Accounting for the effects of lipids in stable isotope ($\delta^{13}C$ and $\delta^{15}N$ values) analysis of skin and blubber of balaenopterid whales. Rapid Commun Mass Spectrom 26:2745–54

Ryan PG, Bester MN (2008). Pelagic predators. In Chown SL, Froneman PW (eds) The Prince Edward Islands. Land-Sea Interactions in a Changing Ecosystem. Sun Press, Stellenbosch

Springer AM, Estes JA, Vliet GB van, Williams TM, Doak DF, Danner EM, Forney KA, Pfister B (2003) Sequential megafaunal collapse in the North Pacific Ocean: An ongoing legacy of industrial whaling? Proc Natl Acad Sci 100:12223–12228

St. Aubin DJ, Smith TG, Geraci JR (1990) Seasonal epidermal molt in beluga whales, *Delphinapterus leucas.* Can J Zool 68:359–367

Tixier P, Authier M, Gasco N, Guinet C (2015) Influence of artificial food provisioning from fisheries on killer whale reproductive output. Anim Conserv 18:207–218

Tixier P, Gasco N, Guinet C (2014) Killer whales of the Crozet Islands: photo-identification catalogue 2014. Centre d'Etudes Biologiques de Chizé – Centre National de la Recherche Scientifique, Villiers en Bois, doi:10.6084/m9.figshare.1060247

Tollit DJ, Pierce GJ, Hobson KA, Bowen WD, Iverson SJ (2010) Diet. In: Boyd IL, Bowen WD, Iverson SJ (eds) Marine Mammal Ecology and Conservation: A Handbook of Techniques. Oxford University Press, Oxford, p 191–221

Trull TW, Armand L (2001) Insights into Southern Ocean carbon export from the $\delta^{13}C$ of particles and dissolved inorganic carbon during the SOIREE iron release experiment. Deep-Sea Res II 48:2655–2680

Walters A (2014) Quantifying the trophic linkage of Antarctic marine predators. PhD thesis, University of Tasmania, Hobart

Williams AJ, Petersen SL, Goren M, Watkins BP (2009) Sightings of killer whales *Orcinus orca* from longline vessels in South African waters, and consideration of the regional conservation status. African J Mar Sci 31:81–86

Williams TM, Estes JA, Doak DF, Springer AM (2004) Killer appetites: Assessing the role of predators in ecological communities. Ecology 85:3373–3384

TABLES

Table 1

$\delta^{13}C$ and $\delta^{15}N$ values of skin collected via remote biopsy sampling from killer whales at Marion Island, before and after we extracted lipids. Social units follow Reisinger (2015). Class: AM – adult male; AF – adult female; SA – subadult. U – Unknown ID. C:N is the atomic carbon:nitrogen ratio. The average values of sample RR-19 and RR-20 were used for analysis.

Social unit	Individual	Class	Sample	Date	Non lipid extracted			Lipid extracted		
					$\delta^{13}C$	$\delta^{15}N$	C:N	$\delta^{13}C$	$\delta^{15}N$	C:N
A	M001	AM	RR-02	2011/08/23	-20.6	12.9	4.1	-18.9	13.0	3.5
A	M001	AM	RR-10	2011/10/22	-20.1	12.9	4.3	-18.5	13.2	3.4
A	M001	AM	DC-04	2012/10/17	-23.6	12.3	7.6	-18.0	14.1	3.4
A	M002	AF	RR-03	2011/09/06	-19.6	11.4	4.1	-18.4	11.7	3.5
A	M027	SA	RR-21	2011/12/14				-18.6	12.4	3.6
B	M004	AF	RR-08	2011/10/12	-19.9	13.4	4.2	-18.4	12.9	3.4
B	M007	AM	RR-01	2011/08/18	-21.2	13.0	4.2	-18.8	13.1	3.5
B	M028	AF	RR-25	2012/05/06	-19.9	11.8	4.4	-18.1	12.4	3.5
C	M005	AM	RR-05	2011/09/20	-20.3	12.2	4.1	-19.2	12.5	3.5
C	M013	AF	RR-17	2011/12/01	-20.0	12.1	4.1	-19.8	13.1	3.7
C	M013	AF	DC-06	2013/05/03	-21.6	11.6	5.6	-18.3	12.2	3.5
C	M015	AF	RR-06	2011/09/20	-20.7	12.1	4.5	-18.9	13.3	3.5
C	M015	AF	DC-07	2013/05/03	-20.4	11.6	4.7	-18.3	12.2	3.5
D	M008	AM	RR-14	2011/11/28	-19.2	13.2	3.9	-19.2	13.4	3.6
D	M008	AM	DC-05	2013/02/07	-20.4	13.0	4.4	-18.6	13.4	3.5
D	M009	SA	RR-22	2011/12/14	-19.3	13.5	4.0	-18.3	13.6	3.5
D	M033	AF	RR-18	2011/12/14	-20.0	12.9	4.2	-18.3	14.6	3.4
E	M031	AF	RR-09	2011/10/19	-21.5	12.3	5.0	-18.7	12.4	3.5
E	M031	AF	RR-11	2011/11/10	-20.5	12.2	4.3	-19.0	12.3	3.5
E	M040	SA	RR-04	2011/09/16	-21.3	11.9	4.7	-18.6	13.0	3.4
E	M050	SA	RR-23	2011/12/18	-20.7	11.8	4.5	-19.3	11.8	3.5
E	M051	SA	RR-13	2011/11/27	-21.3	12.5	5.0	-18.9	12.3	3.5
F	M012	AF	RR-19	2011/12/14	-20.1	11.7	4.3	-18.6	11.9	3.6
F	M012	AF	RR-20	2011/12/14				-18.3	12.4	3.5
F	M025	SA	RR-16	2011/11/29	-19.6	11.8	4.1	-18.4	12.4	3.5
F	M025	SA	DC-02	2012/08/28	-19.6	12.2	4.3	-18.5	12.7	3.5
F	M026	AF	DC-03	2012/10/09	-19.4	11.6	4.2	-18.1	12.1	3.5
G	M016	AM	RR-24	2012/01/03	-19.8	13.5	4.2	-19.3	13.8	3.5
H	M029	AF	RR-12	2011/11/26	-20.7	11.9	4.9	-18.5	12.2	3.5
H	M041	SA	DC-01	2012/08/05	-20.2	12.1	4.6	-18.0	12.7	3.5
-	U	SA	RR-07	2011/09/22	-19.6	12.1	4.1	-18.7	12.1	3.5
-	U	SA	RR-15	2011/11/28	-21.0	12.3	4.6	-19.2	12.6	3.5
n	24				30	30	30	32	32	32
Mean					-20.4	12.3	4.5	-18.6	12.8	3.5
SD					0.9	0.6	0.7	0.4	0.7	0.1

Table 2

Mean (±SD) $\delta^{13}C$ and $\delta^{15}N$ values (‰) of putative killer whale prey at Marion Island. Individual values (and further details, including C:N ratios) are given in Supplementary Tables S1-S3. RBC — red blood cells. [a] Values for rockhopper and macaroni penguins were combined for display (see text, Supplementary Table S1). [b] Values for adult female, subadult, yearling and underyearling elephant seals were combined for display (see text, Supplementary Table S2).

Prey	Abbreviation	*n*	Tissue	Non lipid extracted		Lipid extracted		Adjusted	
				$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$
Aptenodytes patagonicus	KP	8	RBC	-22.7 ± 0.1	10.4 ± 0.3	-	-	-22.3 ± 0.1	10.5 ± 0.3
Eudyptes spp.[a]	EP	12	RBC	-22.9 ± 0.3	8.7 ± 0.6	-	-	-22.5 ± 0.3	8.8 ± 0.6
Arctocephalus tropicalis	SFS	13	Whole blood	-20.1 ± 0.5	11.4 ± 0.3	-	-	-20.5 ± 0.5	12.1 ± 0.3
Mirounga leonina[b]	SES	20	Hair	-19.4 ± 0.7	12.0 ± 0.5	-	-	-20.9 ± 0.7	11.4 ±0.5
Mirounga leonina adult males	SES m	5	Hair	-18.3 ± 1.0	13.3 ± 0.6	-	-	-19.8 ± 1.0	12.7 ± 0.6
Dissostichus eleginoides	PT	10	Muscle	-22.1 ± 1.1	12.7 ± 0.6	-20.2 ± 0.6	13.3 ± 1.1	-	-

Table 3

Values (± SD) used in this study to adjust δ values of tissue we collected to represent muscle tissue.

Taxon in this study	Tissue collected	Adjustment value		Reference species	Reference
		$\delta^{13}C$	$\delta^{15}N$		
Penguins	Red blood cells	0.4 ± 0.1	0.1 ± 0.2	Dunlin (*Calidris alpina pacifica*)	Evans Ogden et al. 2004
Fur seals	Whole blood	-0.4 ± 0.4	0.7 ± 0.6	Harp seals (*Pagophilus groenlandicus*) Harbour seals (*Phoca vitulina*) Ringed seals (*Phoca hispida*)	Hobson et al. 1996
Elephant seal	Hair	-1.5 ± 0.5	-0.6 ± 0.4	*Pagophilus groenlandicus* *Phoca vitulina* *Phoca hispida*	Hobson et al. 1996

Table 4

Comparison of linear mixed effects models of the relationship between killer whale skin $\delta^{13}C$ values (a) and $\delta^{15}N$ values (b). Three predictors were considered: age-sex class (*Class*), social unit (*Unit*) and sighting rate 75 days prior to sampling (*Rate*). Individual identity was included as a random effect in all models. Models are ranked by their small-sample size Akaike Information Criterion scores (AIC_c). $R^2_{GLMM(c)}$ is the conditional coefficient of determination (Nakagawa & Schielzeth 2013).

Model predictors	$R^2_{GLMM(c)}$	AIC_c	ΔAIC_c	Model weight
a) $\delta^{13}C$				
Rate	0.189	38.4	-	0.751
Null model	0.000	41.8	3.42	0.136
Class + *Rate*	0.241	42.5	4.14	0.095
Class	0.054	45.7	7.39	0.019
Rate + *Unit*	0.561	59.5	21.11	0.000
Unit	0.406	62	23.65	0.000
Class + *Rate* + *Unit* (full model)	0.681	65.6	27.23	0.000
Class + *Unit*	0.503	70.4	32.03	0.000
b) $\delta^{15}N$				
Class + *Rate*	0.467	52.0	-	0.709
Class	0.740	54.9	2.92	0.165
Rate	0.734	56.6	4.62	0.070
Null model	0.770	57.1	5.08	0.056
Unit	0.751	66.9	14.89	0.000
Rate + *Unit*	0.657	71.6	19.64	0.000
Class + *Unit*	0.714	73.9	21.95	0.000
Class + *Rate* + *Unit* (full model)	0.747	78.6	26.59	0.000

FIGURES

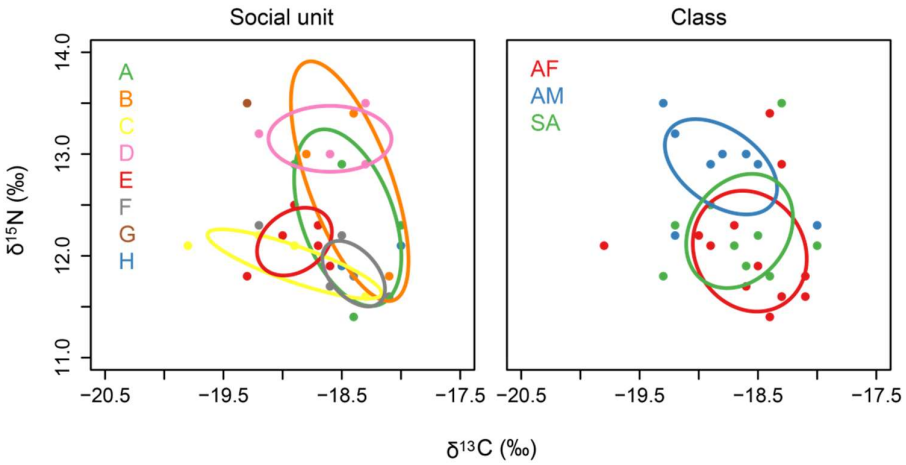

Figure 1

Skin δ values (points) of Marion Island killer whales coloured by social unit (left) and class (right), with small sample size corrected Standard Ellipse Area (SEA_c) (Jackson et al. 2011) superimposed for groups with more than two samples.

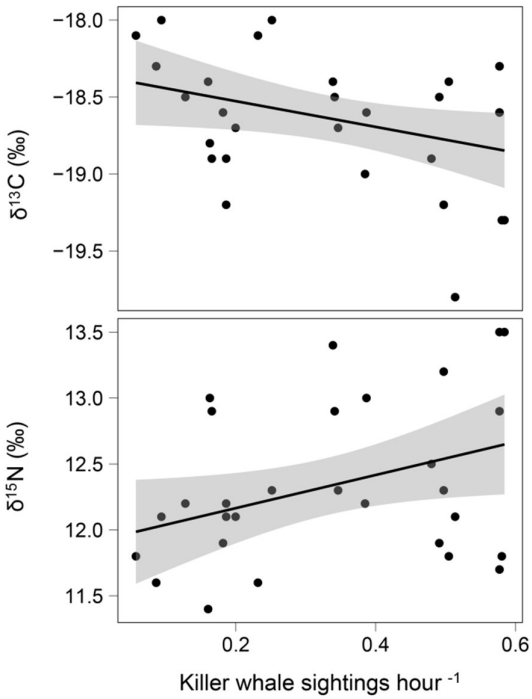

Figure 2

$\delta^{13}C$ (top) and $\delta^{15}N$ (bottom) values in killer whale skin plotted against inshore sighting rate of killer whales in the 75 days prior to sampling. Fitted linear models (lines) are shown with 95% confidence intervals (grey shading).

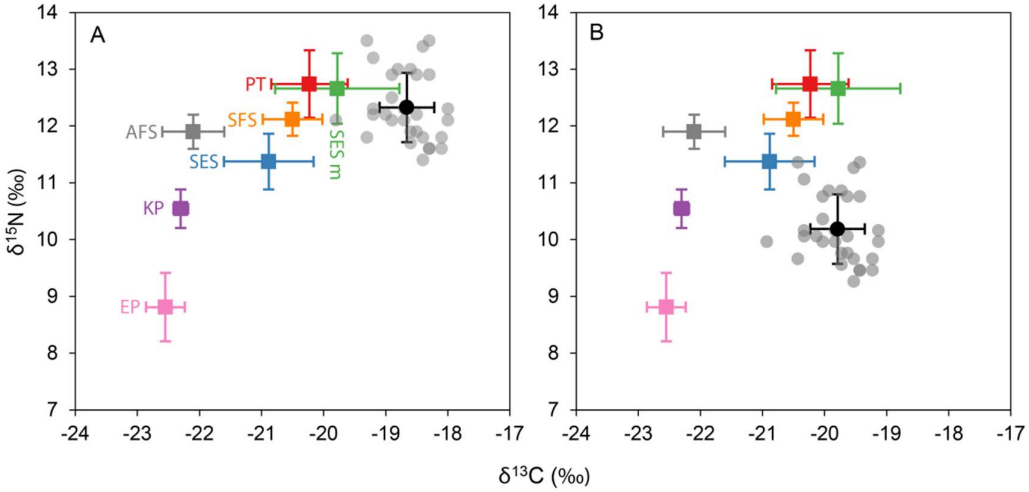

Figure 3

Biplot of $\delta^{13}C$ against $\delta^{15}N$ showing mean values ± SD for killer whales (filled black point) and their prey (squares). Individual values for killer whale skin are displayed as grey points in (a). In (b), discrimination factors have been subtracted from killer whale skin values. PT – Patagonian toothfish; AFS – Antarctic fur seal; SFS – Subantarctic fur seal; SES – southern elephant seal (adult females, subadults and juveniles); SESm – southern elephant seal adult males; KP – king penguin; EP – *Eudyptes* penguins.